

# Package ‘SingleMoleculeFootprinting’

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**Title** Analysis tools for Single Molecule Footprinting (SMF) data

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**Imports** BiocGenerics, Biostrings, BSgenome, GenomeInfoDb,  
GenomicRanges, data.table, grDevices, plyr, IRanges,  
RColorBrewer, stats, QuasR

**Description** SingleMoleculeFootprinting is an R package providing functions to analyze Single Molecule Footprinting (SMF) data. Following the workflow exemplified in its vignette, the user will be able to perform basic data analysis of SMF data with minimal coding effort. Starting from an aligned bam file, we show how to perform quality controls over sequencing libraries, extract methylation information at the single molecule level accounting for the two possible kind of SMF experiments (single enzyme or double enzyme), classify single molecules based on their patterns of molecular occupancy, plot SMF information at a given genomic location

**biocViews** DNAMethylation, Coverage, NucleosomePositioning,  
DataRepresentation, Epigenetics, MethylSeq, QualityControl

**BugReports** <https://github.com/Krebslabrep/SingleMoleculeFootprinting/issues>

**License** GPL-3

**Encoding** UTF-8

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BaitCapture	<i>Bait capture efficiency</i>
-------------	--------------------------------

---

## Description

check bait capture efficiency. Expected to be ~70

## Usage

```
BaitCapture(sampleSheet, genome, baits, clObj = NULL)
```

**Arguments**

sampleSheet	QuasR sample sheet
genome	BS genome
baits	Full path to bed file containing bait coordinates. If chromosome names are in e.g. "1" format, they'll be temporarily converted to "chr1"
clobj	cluster object to emply for parallel processing created using the parallel::makeCluster function. Defaults to NULL

**Value**

bait capture efficiency

**Examples**

```
Qinput = paste0(tempdir(), "/NRF1Pair_Qinput.txt")
library(BSgenome.Mmusculus.UCSC.mm10)

if(file.exists(Qinput)){
  # DO NOT RUN
  # clobj = parallel::makeCluster(5)
  # BaitRegions = SingleMoleculeFootprintingData::EnrichmentRegions_mm10.rds()
  # BaitCaptureEfficiency = BaitCapture(sampleSheet = Qinput, genome = BSgenome.Mmusculus.UCSC.mm10, baits = B
  # parallel::stopCluster(clobj)
}
```

---

BinMethylation

*Summarize methylation inside sorting bins*

---

**Description**

Summarize methylation inside sorting bins

**Usage**

```
BinMethylation(MethSM, TFBS, bin)
```

**Arguments**

MethSM	Single molecule matrix
TFBS	Transcription factor binding site to use for sorting, passed as a GRanges object of length 1
bin	vector of two integers representing the coordinate of a bin relative to the center of the TFBS

**Value**

Reads covering bin with their summarized methylation status

**Examples**

```

Qinput = paste0(tempdir(), "/NRF1Pair_Qinput.txt")
library(BSgenome.Mmusculus.UCSC.mm10)

if(file.exists(Qinput)){
  QuasRprj = GetQuasRprj(Qinput, BSgenome.Mmusculus.UCSC.mm10)

  MySample = readr::read_delim(Qinput, delim = "\t")$SampleName[1]
  Region_of_interest = GRanges(seqnames = "chr6", ranges = IRanges(start = 88106000, end = 88106500), strand = "+")

  Methylation = CallContextMethylation(sampleSheet = Qinput,
                                      sample = MySample,
                                      genome = BSgenome.Mmusculus.UCSC.mm10,
                                      range = Region_of_interest,
                                      coverage = 20,
                                      ConvRate.thr = 0.2)

  TFBSs = GenomicRanges::GRanges("chr6", IRanges(c(88106253), c(88106263)), strand = "-")
  elementMetadata(TFBSs)$name = c("NRF1")
  names(TFBSs) = c(paste0("TFBS_", c(4305216)))

  binMethylationValues = BinMethylation(MethSM = Methylation[[2]], TFBS = TFBSs, bin = c(-15,15))
}

```

---

CallContextMethylation

*Call Context Methylation*

---

**Description**

Can deal with multiple samples

**Usage**

```

CallContextMethylation(
  sampleSheet,
  sample,
  genome,
  range,
  coverage = 20,
  ConvRate.thr = 0.2,
  verbose = TRUE
)

```

**Arguments**

sampleSheet	QuasR pointer file
sample	for now this works for sure on one sample at the time only
genome	BSgenome
range	GenimocRange representing the genomic region of interest

coverage	coverage threshold. Defaults to 20.
ConvRate.thr	Conversion rate threshold. Double between 0 and 1, defaults to 0.2
verbose	TRUE/FALSE

**Value**

List with two Granges objects: average methylation call (GRanges) and single molecule methylation call (matrix)

**Examples**

```
Qinput = paste0(tempdir(), "/NRF1Pair_Qinput.txt")
library(BSgenome.Mmusculus.UCSC.mm10)

if(file.exists(Qinput)){
  QuasRprj = GetQuasRprj(Qinput, BSgenome.Mmusculus.UCSC.mm10)

  MySample = readr::read_delim(Qinput, delim = "\t")$SampleName[1]
  Region_of_interest = GRanges(seqnames = "chr6", ranges = IRanges(start = 88106000, end = 88106500), strand = "+")

  Methylation = CallContextMethylation(sampleSheet = Qinput,
                                       sample = MySample,
                                       genome = BSgenome.Mmusculus.UCSC.mm10,
                                       range = Region_of_interest,
                                       coverage = 20,
                                       ConvRate.thr = 0.2)
}
```

---

CollapseStrands

*Collapse strands*


---

**Description**

Collapse strands

**Usage**

```
CollapseStrands(MethGR, context, verbose = TRUE)
```

**Arguments**

MethGR	Granges obj of average methylation
context	"GC" or "CG". Broad because indicates just the directionality of collapse.
verbose	TRUE/FALSE

**Value**

MethGR with collapsed strands (everything turned to - strand)

---

CollapseStrandsSM      *Collapse strands in single molecule matrix*

---

### Description

The idea here is that (regardless of context) if a C is on the - strand, calling getSeq on that coord (N.b. unstranded, that's the important bit) will give a "G", a "C" if it's a + strand.

### Usage

```
CollapseStrandsSM(MethSM, context, genome, chr, verbose = TRUE)
```

### Arguments

MethSM	Single molecule matrix
context	"GC" or "CG". Broad because indicates just the directionality of collapse.
genome	BSgenome
chr	Chromosome, MethSM doesn't carry this info
verbose	TRUE/FALSE

### Value

Strand collapsed MethSM

---

ConversionRate      *Conversion rate*

---

### Description

calculate sequencing library conversion rate on a chromosome of choice

### Usage

```
ConversionRate(sampleSheet, genome, chr = 19, clObj = NULL)
```

### Arguments

sampleSheet	QuasR sample sheet
genome	BS genome
chr	chromosome to calculate conversion rate on (default: 19)
clObj	cluster object to emply for parallel processing created using the parallel::makeCluster function. Defaults to NULL

### Value

Conversion rate

**Examples**

```

Qinput = paste0(tempdir(), "/NRF1Pair_Qinput.txt")
library(BSgenome.Mmusculus.UCSC.mm10)

if(file.exists(Qinput)){
  # DO NOT RUN
  # clObj = parallel::makeCluster(5)
  # ConversionRatePrecision = ConversionRate(sampleSheet = Qinput, genome = BSgenome.Mmusculus.UCSC.mm10, chr
  # parallel::stopCluster(clObj)
}

```

---

CoverageFilter      *Filter Cs for coverage*

---

**Description**

Filter Cs for coverage

**Usage**

```
CoverageFilter(MethGR, thr)
```

**Arguments**

MethGR	Granges obj of average methylation
thr	converage threshold

**Value**

filtered MethGR

---

DetectExperimentType      *Detect type of experiment*

---

**Description**

Detect type of experiment

**Usage**

```
DetectExperimentType(Samples, verbose = TRUE)
```

**Arguments**

Samples	SampleNames field from QuasR sampleSheet
verbose	TRUE/FALSE

**Value**

String indicating the type of experiment detected

**Examples**

```
Qinput = paste0(tempdir(), "/NRF1Pair_Qinput.txt")

if(file.exists(Qinput)){
  sample = readr::read_delim(Qinput, delim = "\t")$SampleName
  ExpType = DetectExperimentType(sample)
}
```

---

**FilterByConversionRate**

*Calculate reads conversion rate*

---

**Description**

Calculate reads conversion rate

**Usage**

```
FilterByConversionRate(MethSM, chr, genome, thr = 0.2, verbose = TRUE)
```

**Arguments**

MethSM	as comes out of the func GetSingleMolMethMat
chr	Chromosome, MethSM doesn't carry this info
genome	BSgenome
thr	Double between 0 and 1. Threshold above which to filter reads. Defaults to 0.2
verbose	TRUE/FALSE

**Value**

Filtered MethSM

**Examples**

```
Qinput = paste0(tempdir(), "/NRF1Pair_Qinput.txt")
library(BSgenome.Mmusculus.UCSC.mm10)

if(file.exists(Qinput)){
  QuasRprj = GetQuasRprj(Qinput, BSgenome.Mmusculus.UCSC.mm10)

  sample = readr::read_delim(Qinput, delim = "\t")$SampleName
  range = GRanges(seqnames = "chr6", ranges = IRanges(start = 88106000, end = 88106500), strand = "*")

  MethSM = GetSingleMolMethMat(QuasRprj, range, sample)
  MethSM = FilterByConversionRate(MethSM, chr = "chr6", genome = BSgenome.Mmusculus.UCSC.mm10, thr = 0.8)
}
```



---

 FilterContextCytosines

*Filter Cytosines in context*


---

**Description**

Filter Cytosines in context

**Usage**

```
FilterContextCytosines(MethGR, genome, context)
```

**Arguments**

MethGR	Granges obj of average methylation
genome	BSgenome
context	Context of interest (e.g. "GC", "CG",...)

**Value**

filtered Granges obj

**Examples**

```
Qinput = paste0(tempdir(), "/NRF1Pair_Qinput.txt")
library(BSgenome.Mmusculus.UCSC.mm10)

if(file.exists(Qinput)){
  QuasRprj = GetQuasRprj(Qinput, BSgenome.Mmusculus.UCSC.mm10)

  Samples = readr::read_delim(Qinput, delim = "\t")$SampleName
  sample = Samples[1]
  range = GRanges(seqnames = "chr6", ranges = IRanges(start = 88106000, end = 88106500), strand = "*")

  MethGR = QuasR::qMeth(QuasRprj[grep(sample, Samples)], mode="allC", range, collapseBySample = TRUE, keepZero = TRUE)
  FilterContextCytosines(MethGR, BSgenome.Mmusculus.UCSC.mm10, "NGCNN")
}
```

---

 FixOverhang

*Fixing overhang before stand collapsing*


---

**Description**

Fixing overhang before stand collapsing

**Usage**

```
FixOverhang(MethGR, context, which)
```

**Arguments**

MethGR	Granges obj of average methylation
context	context
which	"Top" "Bottom"

**Value**

MethGR with fixed overhang

---

GetQuasRprj	<i>Get QuasRprj</i>
-------------	---------------------

---

**Description**

Get QuasRprj

**Usage**

```
GetQuasRprj(sampleSheet, genome)
```

**Arguments**

sampleSheet	QuasR pointer file
genome	BSgenome

**Value**

QuasR project object as returned by QuasR::qAlign function

**Examples**

```
Qinput = paste0(tempdir(), "/NRF1Pair_Qinput.txt")
library(BSgenome.Mmusculus.UCSC.mm10)

if(file.exists(Qinput)){
  QuasRprj = GetQuasRprj(Qinput, BSgenome.Mmusculus.UCSC.mm10)
}
```

---

GetSingleMolMethMat     *Get Single Molecule methylation matrix*

---

**Description**

Used internally as the first step in CallContextMethylation

**Usage**

```
GetSingleMolMethMat(QuasRprj, range, sample)
```

**Arguments**

QuasRprj	QuasR project object as returned by calling the QuasR function qAlign on previously aligned data
range	GenimocRange representing the genomic region of interest
sample	One of the sample names as reported in the SampleName field of the QuasR pointer file provided to qAlign. N.b. all the files with the passed sample name will be used to call methylation

**Value**

Single molecule methylation matrix (all Cytosines)

**Examples**

```
Qinput = paste0(tempdir(), "/NRF1Pair_Qinput.txt")
library(BSgenome.Mmusculus.UCSC.mm10)

if(file.exists(Qinput)){
  QuasRprj = GetQuasRprj(Qinput, BSgenome.Mmusculus.UCSC.mm10)

  sample = readr::read_delim(Qinput, delim = "\t")$SampleName
  range = GRanges(seqnames = "chr6", ranges = IRanges(start = 88106000, end = 88106500), strand = "*")

  MethSM = GetSingleMolMethMat(QuasRprj, range, sample)
}
```

---

HierarchicalClustering

*Perform Hierarchical clustering on single reads*

---

**Description**

Perform Hierarchical clustering on single reads

**Usage**

```
HierarchicalClustering(MethSM)
```

**Arguments**

MethSM            Single molecule methylation matrix

**Value**

Single molecule matrix after hierarchical clustering

---

OneTFstates            *Design states for single TF case*

---

**Description**

Design states for single TF case

**Usage**

OneTFstates()

**Value**

list of states

---

PlotAvgSMF            *Plot average methylation*

---

**Description**

Plot average methylation

**Usage**

PlotAvgSMF(MethGR, range, TFBSs)

**Arguments**

MethGR            Average methylation GRanges obj  
 range            GRanges interval to plot  
 TFBSs            GRanges object of transcription factor binding sites to include in the plot. Assumed to be already subset.

**Value**

Average SMF signal at single site

**Examples**

```

Qinput = paste0(tempdir(), "/NRF1Pair_Qinput.txt")
library(BSgenome.Mmusculus.UCSC.mm10)

if(file.exists(Qinput)){
  QuasRprj = GetQuasRprj(Qinput, BSgenome.Mmusculus.UCSC.mm10)

  MySample = readr::read_delim(Qinput, delim = "\t")$SampleName[1]
  Region_of_interest = GRanges(seqnames = "chr6", ranges = IRanges(start = 88106000, end = 88106500), strand = "+")

  Methylation = CallContextMethylation(sampleSheet = Qinput,
                                       sample = MySample,
                                       genome = BSgenome.Mmusculus.UCSC.mm10,
                                       range = Region_of_interest,
                                       coverage = 20,
                                       ConvRate.thr = 0.2)

  TFBSs = GenomicRanges::GRanges("chr6", IRanges(c(88106253), c(88106263)), strand = "-")
  elementMetadata(TFBSs)$name = c("NRF1")
  names(TFBSs) = c(paste0("TFBS_", c(4305216)))

  PlotAvgSMF(MethGR = Methylation[[1]], range = Region_of_interest, TFBSs = TFBSs)
}

```

---

PlotSingleMoleculeStack

*Plot single molecule stack*


---

**Description**

Plot single molecule stack

**Usage**

```
PlotSingleMoleculeStack(MethSM, range)
```

**Arguments**

MethSM	Single molecule methylation matrix
range	GRanges interval to plot

**Value**

Single molecule plot

---

PlotSingleSiteSMF      *Plot SMF data at single site*

---

### Description

Plot SMF data at single site

### Usage

```
PlotSingleSiteSMF(
  ContextMethylation,
  sample,
  range,
  SortedReads = NULL,
  TFBSs,
  saveAs = NULL
)
```

### Arguments

ContextMethylation	Context methylation object as returned by CallContextMethylation function
sample	one sample as reported in the SampleName files of the QuasR sampleSheet
range	GRange interval to plot
SortedReads	Defaults to NULL, in which case will plot unsorted reads. Sorted reads object as returned by SortReads function or "HC" to perform hierarchical clustering
TFBSs	GRange or GRangesList of transcription factor binding sites to add to the plot. If SortedReads are passed, the format of TFBSs (GRanges vs GRangesList) will be used to determine if single molecules were sorted based on one or multiple TFs
saveAs	Full path to pdf file to save plot to. Defaults to NULL, in which case will only display

### Value

Single site plot including average SMF signal, single molecules stack and state quantification plot

### Examples

```
Qinput = paste0(tempdir(), "/NRF1Pair_Qinput.txt")
library(BSgenome.Mmusculus.UCSC.mm10)

if(file.exists(Qinput)){
  QuasRprj = GetQuasRprj(Qinput, BSgenome.Mmusculus.UCSC.mm10)

  MySample = readr::read_delim(Qinput, delim = "\t")$SampleName[1]
  Region_of_interest = GRanges(seqnames = "chr6", ranges = IRanges(start = 88106000, end = 88106500), strand =

  Methylation = CallContextMethylation(sampleSheet = Qinput,
                                       sample = MySample,
                                       genome = BSgenome.Mmusculus.UCSC.mm10,
```



```

        genome = BSgenome.Mmusculus.UCSC.mm10,
        range = Region_of_interest,
        coverage = 20,
        ConvRate.thr = 0.2)

    PlotSM(MethSM = Methylation[[2]], range = Region_of_interest)
}

```

---

SampleCorrelation      *Intersample correlation*

---

### Description

pair plot of sample correlations

### Usage

```
SampleCorrelation(samples, context, CellType)
```

### Arguments

samples	Avg methylation object. Can also be set to "Example" to produce plot using example data of the kind specified by the @param CellType
context	one of "AICs", "DGCHN", "NWCGW". The first should be chosen for TKO experiments. For experiments carried on WT cells, we recommend checking both the "DGCHN" and "NWCGW" contexts by running this function once per context.
CellType	Cell type to compare your samples to. At the moment, this can be one of "ES", "NP", "TKO".

### Value

Inter-sample correlation plot

---

SingleTFStateQuantificationPlot  
*Single TF state quantification bar*

---

### Description

Single TF state quantification bar

### Usage

```
SingleTFStateQuantificationPlot(states, OrderedReads)
```

### Arguments

states	as returned by OneTFstates function
OrderedReads	Reads ordered by states



**Value**

single TF state quantification plot

---

SortReads	<i>Sort reads by single TF</i>
-----------	--------------------------------

---

**Description**

Sort reads by single TF

**Usage**

```
SortReads(MethSM, TFBS, BinsCoord, SortByCluster)
```

**Arguments**

MethSM	Single molecule matrix
TFBS	Transcription factor binding site to use for sorting
BinsCoord	list of 3 bin coordinates relative to the center of the TFBS.
SortByCluster	T/F

**Value**

list of sorted reads

**Examples**

```
Qinput = paste0(tempdir(), "/NRF1Pair_Qinput.txt")
library(BSgenome.Mmusculus.UCSC.mm10)

if(file.exists(Qinput)){
  QuasRprj = GetQuasRprj(Qinput, BSgenome.Mmusculus.UCSC.mm10)

  MySample = readr::read_delim(Qinput, delim = "\t")$SampleName[1]
  Region_of_interest = GRanges(seqnames = "chr6", ranges = IRanges(start = 88106000, end = 88106500), strand = "+")

  Methylation = CallContextMethylation(sampleSheet = Qinput,
                                       sample = MySample,
                                       genome = BSgenome.Mmusculus.UCSC.mm10,
                                       range = Region_of_interest,
                                       coverage = 20,
                                       ConvRate.thr = 0.2)

  TFBSs = GenomicRanges::GRanges("chr6", IRanges(c(88106253), c(88106263)), strand = "-")
  elementMetadata(TFBSs)$name = c("NRF1")
  names(TFBSs) = c(paste0("TFBS_", c(4305216)))
  BinsCoord = list(c(-35,-25), c(-15,15), c(25,35))

  SortedReads = SortReads(Methylation[[2]], TFBSs, BinsCoord, SortByCluster = FALSE)
}
```

---

SortReadsBySingleTF     *Wrapper to SortReads for single TF case*

---

## Description

Wrapper to SortReads for single TF case

## Usage

```
SortReadsBySingleTF(MethSM, TFBS)
```

## Arguments

MethSM	Single molecule matrix
TFBS	Transcription factor binding site to use for sorting, passed as a GRanges object of length 1

## Value

List of reads sorted by single TF

## Examples

```
Qinput = paste0(tempdir(), "/NRF1Pair_Qinput.txt")
library(BSgenome.Mmusculus.UCSC.mm10)

if(file.exists(Qinput)){
  QuasRprj = GetQuasRprj(Qinput, BSgenome.Mmusculus.UCSC.mm10)

  MySample = readr::read_delim(Qinput, delim = "\t")$SampleName[1]
  Region_of_interest = GRanges(seqnames = "chr6", ranges = IRanges(start = 88106000, end = 88106500), strand = "+")

  Methylation = CallContextMethylation(sampleSheet = Qinput,
                                       sample = MySample,
                                       genome = BSgenome.Mmusculus.UCSC.mm10,
                                       range = Region_of_interest,
                                       coverage = 20,
                                       ConvRate.thr = 0.2)

  TFBSs = GenomicRanges::GRanges("chr6", IRanges(c(88106253), c(88106263)), strand = "-")
  elementMetadata(TFBSs)$name = c("NRF1")
  names(TFBSs) = c(paste0("TFBS_", c(4305216)))

  SortedReads = SortReadsBySingleTF(MethSM = Methylation[[2]], TFBS = TFBSs)
}
```

---

SortReadsByTFCluster *Wrapper to SortReads for TF cluster case*

---

## Description

Wrapper to SortReads for TF cluster case

## Usage

```
SortReadsByTFCluster(MethSM, TFBSs)
```

## Arguments

MethSM	Single molecule matrix
TFBSs	Transcription factor binding sites to use for sorting, passed as a GRanges object of length > 1

## Value

List of reads sorted by TF cluster

## Examples

```
Qinput = paste0(tempdir(), "/NRF1Pair_Qinput.txt")
library(BSgenome.Mmusculus.UCSC.mm10)

if(file.exists(Qinput)){
  QuasRprj = GetQuasRprj(Qinput, BSgenome.Mmusculus.UCSC.mm10)

  MySample = readr::read_delim(Qinput, delim = "\t")$SampleName[1]
  Region_of_interest = GRanges(seqnames = "chr6", ranges = IRanges(start = 88106000, end = 88106500), strand = "+")

  Methylation = CallContextMethylation(sampleSheet = Qinput,
                                       sample = MySample,
                                       genome = BSgenome.Mmusculus.UCSC.mm10,
                                       range = Region_of_interest,
                                       coverage = 20,
                                       ConvRate.thr = 0.2)

  TFBSs = GenomicRanges::GRanges("chr6", IRanges(c(88106253), c(88106263)), strand = "-")
  elementMetadata(TFBSs)$name = c("NRF1")
  names(TFBSs) = c(paste0("TFBS_", c(4305216)))

  SortedReads = SortReadsByTFCluster(MethSM = Methylation[[2]], TFBSs = TFBSs)
}
```

---

 StateQuantificationPlot

*Plot states quantification bar*


---

### Description

Plot states quantification bar

### Usage

```
StateQuantificationPlot(SortedReads)
```

### Arguments

SortedReads      Sorted reads object as returned by SortReads function

### Value

Bar plot quantifying states

### Examples

```
Qinput = paste0(tempdir(), "/NRF1Pair_Qinput.txt")
library(BSgenome.Mmusculus.UCSC.mm10)

if(file.exists(Qinput)){
  QuasRprj = GetQuasRprj(Qinput, BSgenome.Mmusculus.UCSC.mm10)

  MySample = readr::read_delim(Qinput, delim = "\t")$SampleName[1]
  Region_of_interest = GRanges(seqnames = "chr6", ranges = IRanges(start = 88106000, end = 88106500), strand = "+")

  Methylation = CallContextMethylation(sampleSheet = Qinput,
                                       sample = MySample,
                                       genome = BSgenome.Mmusculus.UCSC.mm10,
                                       range = Region_of_interest,
                                       coverage = 20,
                                       ConvRate.thr = 0.2)

  TFBSs = GenomicRanges::GRanges("chr6", IRanges(c(88106253), c(88106263)), strand = "-")
  elementMetadata(TFBSs)$name = c("NRF1")
  names(TFBSs) = c(paste0("TFBS_", c(4305216)))

  SortedReads = SortReadsByTFCluster(MethSM = Methylation[[2]], TFBSs = TFBSs)
  StateQuantificationPlot(SortedReads = SortedReads)
}
```

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TFPairStateQuantificationPlot  
*TF pair state quantification bar*

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**Description**

TF pair state quantification bar

**Usage**

TFPairStateQuantificationPlot(states, OrderedReads)

**Arguments**

states            as returned by TFpairStates function  
OrderedReads    Reads ordered by states

**Value**

TF pair state quantification plot

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TFpairStates            *Design states for TF pair case*

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**Description**

Design states for TF pair case

**Usage**

TFpairStates()

**Value**

list of states

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